



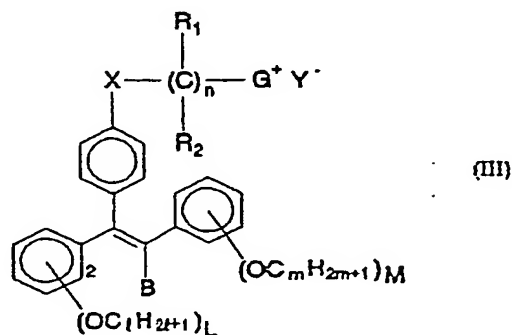
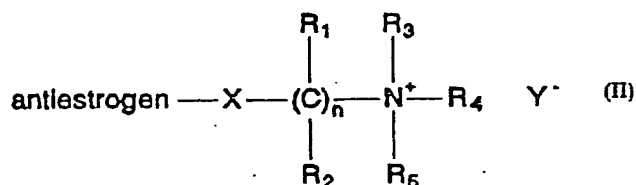
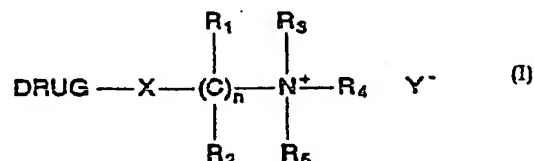
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/14, 31/40, C07C 217/18, 217/20, C07D 207/08		A1	(11) International Publication Number: WO 95/26720
			(43) International Publication Date: 12 October 1995 (12.10.95)
(21) International Application Number: PCT/US95/03941		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KE, KG, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
(22) International Filing Date: 31 March 1995 (31.03.95)			
(30) Priority Data: 223,074 4 April 1994 (04.04.94) US			
(60) Parent Application or Grant (63) Related by Continuation US 223,074 (CIP) Filed on 4 April 1994 (04.04.94)		Published With international search report. With amended claims.	
(71) Applicant (for all designated States except US): PHARMOS CORPORATION [US/US]; 101 East 52nd Street, New York, NY 10022 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): BIEGON, Anat (IL/IL); 7 Hameiri Street, Neit Afeka, 69413 Tel Aviv (IL). BREWSTER, Marcus [US/US]; 6705 S.W. 44th Avenue, Gainesville, FL 32608 (US).			
(74) Agents: FANUCCI, Allan, A. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).			

(54) Title: PERMANENTLY IONIC DERIVATIVES OF STEROID HORMONES AND THEIR ANTAGONISTS

(57) Abstract

The present disclosure relates to compounds of general formulae (I), (II) and (III), wherein DRUG is a steroid agonist or antagonist, a mixed agonist-antagonist, or a partial agonist, and to the use of such compounds as anti-inflammatory and anti-tumor agents.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

**PERMANENTLY IONIC DERIVATIVES
OF STEROID HORMONES AND THEIR ANTAGONISTS**

5

FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions that are suitable for use in methods of treatment requiring steroid hormones or their antagonists, and to novel permanently ionic chemical compounds that may be used in such methods.

15

BACKGROUND OF THE INVENTION

In principle, certain types of steroid hormones used in therapeutic procedures might beneficially be excluded from entering the central nervous system (CNS). It would be advantageous to produce derivatives of such hormones that retain their action in peripheral tissues and organs but are devoid of CNS activity. Penetration into the CNS requires that a compound be sufficiently lipophilic to cross the blood brain barrier (BBB). Therefore, to prevent penetration across the BBB it will often suffice to create an ionic drug derivative, especially a derivative that has a large charged moiety attached. However, the receptors through which steroid hormones exert their action are intracellular. Thus, it is not at all obvious that a derivative which is incapable of penetrating the BBB can in fact cross the cell membrane to reach the appropriate receptors. In other words, the cell membrane also constitutes a lipophilic barrier which hinders the passage of charged molecules.

Examples of categories of steroid receptor binding drugs that would beneficially be excluded from the CNS include: corticosteroids, which have been shown to produce neuronal loss; progestins, which are used as an adjunct to estrogen replacement therapy in order to prevent endometrial hyperplasia; and antiestrogens that are used predominantly in preventing or retarding the growth of tumors.

The use of progestins as an adjunct to estrogen in hormone replacement therapy in peri- or postmenopausal women is predicated on their opposition to the effects of estrogen. While estrogen has highly desirable actions in the brain, bone, and cardiovascular system, unopposed estrogen may be undesirable, particularly for the endometrial lining of the uterus. Progestins effectively prevent the undesirable hyperplasia of the endometrium. However, in the CNS they induce depression and hot flushes by virtue of their antiestrogenic activity. The use of progestins limited to their peripheral activity would be advantageous.

Corticosteroids are extremely useful in suppressing inflammatory reactions. Their clinical use is severely curtailed by undesirable side effects, especially during chronic administration (Sapolsky et al. (1985) *J. Neurosci.* 5, 1222-1227; Landfield (1987) *Prog. Brain Res.* 72, 279-300). Many of these adverse side effects could be avoided if these compounds were incapable of exerting their harmful action in the CNS.

Pharmaceutical therapy for breast cancer consists currently of cytotoxic and hormonal agents. Hormonal therapy was developed because, in many women, the breast cancer cells have receptors for the steroid hormone estrogen. The growth of these estrogen receptor-positive cancer cells can be stimulated by

estrogen. Antiestrogen therapy attempts to reduce or stop the synthesis of estrogen or to block the action of estrogen on the cancer cell.

Among the hormonals, tamoxifen (U.S. Patent No. 4,536,516) holds a preeminent position. Originally designed as an antiestrogen to treat breast cancer in patients with estrogen receptor-positive tumors, the drug was also found to slow the growth of breast cancer in women with estrogen receptor-negative tumors. Tamoxifen is, therefore, useful in most patients. The antiestrogen tamoxifen is particularly effective in delaying recurrence in breast cancer patients and in the palliative treatment of advanced metastatic breast cancer. It is also useful in the treatment of additional types of cancer including prostatic neoplasms (Litherland, S. et al. Cancer Treatment Reviews, 1988, 15: 183; Jordan, C., Br. J. Pharmacol., 1993, 110: 507).

Antiestrogens, including tamoxifen, compete with estrogen for receptor sites in cancerous tissues. Occupancy of the receptor site by an antiestrogen fails to elicit the further transcriptional actions generated by estrogens and blocks their activity. It is generally believed that estrogens function by binding to the target cell cytosolic receptors then moving into the cell nucleus and in turn affecting DNA transcription.

Tamoxifen and other antiestrogens also affect cellular, tumor, and organ responses by less direct mechanisms. Antiestrogens penetrate into the CNS and disrupt the normal feedback loops for hormonal balance (hypothalamus-pituitary axis) by blockading estrogen receptors in the anterior pituitary and hypothalamus. Often the physiological activity arising from altered circulating hormone levels is undesirable and leads to

a variety of known side effects of antiestrogen administration. Hot flushes, which are CNS-mediated, are the most common side effect of tamoxifen (Jordan, C., *ibid.*).

5 The actions of tamoxifen and other nonsteroidal antiestrogens are complicated further by their mixed agonist-antagonist nature. Tamoxifen has partial agonist (estrogenic) activity, and the degree of agonist versus antagonist (antiestrogenic) activity is
10 a function of the target cell (Furr, B. et al., *Pharmacology & Therapeutics*, 1984, 24: 127). Tamoxifen has been shown to act mainly as an antagonist in breast and brain, while its agonistic activity is more apparent in bone and the
15 cardiovascular system.

Whereas it has been postulated that pure antiestrogenic compounds might be more effective antitumor agents, another school of thought asserts that it is advantageous to retain the partial
20 estrogenic activity of these antitumor agents since agonistic estrogenic activity is of proven value in preventing osteoporosis, cardiovascular disorders, and postmenopausal symptoms such as hot flushes (Jordan, C., *Br. J. Pharmacol.*, 1993, 110: 507) and possibly
25 age-related cognitive decline and depression (Sherwin, B., *Psychoneuroendocrinology*, 1988, 13: 345). In particular, it has been envisaged that antiestrogen therapy could be administered prophylactically to healthy women at high risk for developing breast
30 cancer, and large prospective clinical trials are underway to test this concept. It would be very desirable to minimize the deleterious effects of estrogen deprivation (or antagonism) in this population.

Considerable effort has been invested in the development of novel tamoxifen analogs presumed to have improved therapeutic potential, by virtue of increased selectivity as antiestrogenic compounds (e.g. US 4,973,755; EP 0 168,175) or higher affinity for the estrogen receptor (WO 92/06068).

In various cases there have been discrepancies between the activity of tamoxifen derivatives *in vitro* and *in vivo*. For example, Foster et al. (Anticancer Drug Design, 1986, 1: 245) describes the effect of various tamoxifen hydroxy-derivatives on the growth of MCF-7 breast cancer cell line in culture. Hydroxy tamoxifen derivatives that are highly active *in vitro* were found to be less active than tamoxifen *in vivo* against a DMBA-induced estrogen receptor-positive tumor in rats, and only slightly more active against a hormone dependent mammary tumor in mice. However, when 4-hydroxy-tamoxifen itself is administered *in vivo*, its polarity reduces its ability to cross the cell membrane, thereby reducing its access to estrogen receptors located in the cytoplasm. Indeed, *in vivo* tests indicate a 4-hydroxytamoxifen to be less active than the native tamoxifen (Foster et al., J. Med. Chem., 1985, 28: 1491).

Jarman et al., Anticancer Drug Design, 1986, 1: 259-268 described the preparation and testing of tamoxifen as well as tamoxifen methiodide, ethyl bromide, and N-oxide. When tested *in vitro*, these derivatives were reported not to halt the proliferation of breast tumor cell lines grown in culture. The interpretation offered was that the quaternized analogs fail to enter the cells (Jarman, M. et al. *ibid.*; Canabrana, B., Hidalgo, A. Pharmacology, 1992, 329). It was predicted,

therefore, that these compounds would be of no therapeutic value *in vivo*.

5

SUMMARY OF THE INVENTION

The present invention provides compounds that act as steroid agonists and/or antagonists in peripheral organs and tissues, as required, while virtually
10 devoid of activity in the central nervous system. Contrary to the teachings of the prior art, it is shown herein that compounds that are rendered ionic can achieve their desired therapeutic action in their target cells, even when they may exert their effects
15 intracellularly.

According to the present invention it is now disclosed that, unexpectedly, ionic derivatives of the antiestrogen tamoxifen which were predicted to be of no value *in vivo* on the basis of their lack of
20 activity *in vitro* are in fact more active *in vivo* than the parent compound.

In view of the robust activity of tamoxifen and other antiestrogens in the brain, apart from the disruption of normal feedback loops for gonadotropic
25 function in the hypothalamic and pituitary regions, it is often desirable to use antiestrogenic agents that do not cross into the CNS and brain. Such peripheral antiestrogens would, in general, exhibit reduced side effects during clinical use and particularly in
30 premenopausal women.

Hydrophilic compounds and particularly compounds with ionic charges (cationic or anionic) are often very poorly distributed into the CNS and brain since a lipophilic barrier (the blood-brain barrier or "BBB")
35 exists. One method for creating a permanent charge on

a drug is the incorporation of a quaternary ammonium salt (nitrogen with four carbon atoms attached).

Tamoxifen and other antiestrogens that contain an amino group can be quaternized (converted to a quaternary ammonium group) resulting in a permanent positive charge on the parent molecule which should effectively reduce its penetration across physiological membranes that are inherently lipophilic and resistant to penetration of ions, particularly large ions.

In those cases where the drug contains no appropriate amine group, a bridging group can be utilized in order to provide an ionizable amine or other ionic species. In the case of progestins bearing a hydroxyl group, such as 17-hydroxy progesterone or medroxyprogesterone, a bridging group can be attached in the form of an ester or phosphate or any other suitable species that can provide a permanent ionic moiety. In the case of corticosteroids, the 21-hydroxyl similarly can serve to attach a bridging group for providing an ionic moiety. Thus, by utilizing a strategy involving bridging groups, steroid hormone agonists can be converted to permanently ionic derivatives according to the general precepts of the instant invention.

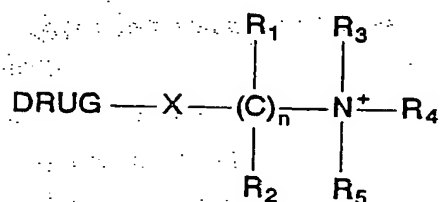
It is an object of this invention to provide peripheral antiestrogens for clinical treatment of cancer and other diseases and pathological conditions. These peripheral antiestrogens will possess estrogen antagonist activity, and may possess partial estrogen agonist or mixed activity, but are limited in biodistribution by being excluded from the CNS and brain, thereby exhibiting reduced side effects and being beneficial for clinical use. Thus, a primary objective of the present invention is to provide novel

compounds that retain antiestrogenic activity in tumor tissue, while rendering them incapable of penetrating into the brain.

Another object of this invention and beneficial to clinical use is the comparatively elevated circulatory levels of these agents due to the fact that they are not sequestered in fat tissue, thereby allowing for more precise control of dosing.

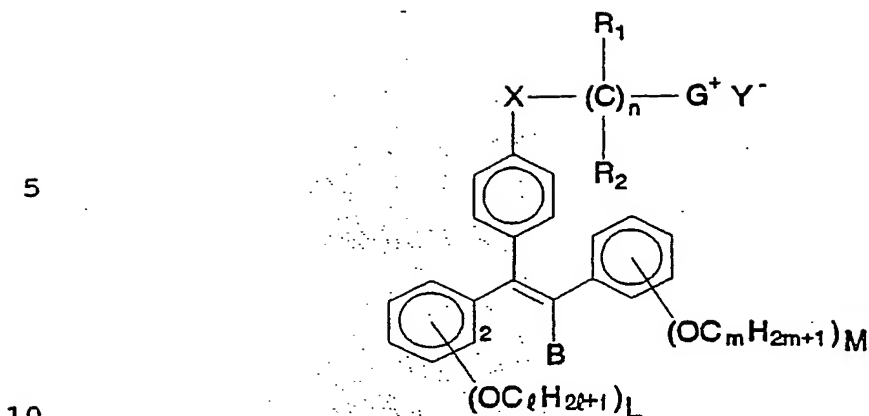
Yet another aspect of this invention is to provide for the formulation and drug delivery of the aforementioned peripheral antiestrogens.

These and other objects of the present invention are achieved by providing compounds of the general formula:



wherein Y is any non-toxic pharmaceutically acceptable anion, DRUG is a steroid antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12, as well as by providing compounds according to the formula

- 9 -



wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H, alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the permanently ionic group G, said moiety being selected from the group consisting of -CH₂C(R₁)(R₂)- and -CH₂-O-; L and M are independently 0-3; l, m, and p are independently 1-7; and Y is a pharmaceutically acceptable anion, provided that when G is -N(R')(R'')(R''') or -(O)N(R')(R''), R' and R'' cannot both be unsubstituted alkyl.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 compares the effects of tamoxifen and of tamoxifen methiodide on uterine weight.

 Figure 2 compares the dose response of tamoxifen and tamoxifen methiodide either alone or in combination with estradiol on creatine kinase activity
10 in the bone and uterus.

 Figure 3 compares the effects of tamoxifen and of tamoxifen methiodide on body weight.

 Figure 4 compares the effects of tamoxifen and of tamoxifen methiodide on breast cancer tumors.

15 Figure 5 shows the dose dependence of the effect of tamoxifen methiodide on breast cancer tumors.

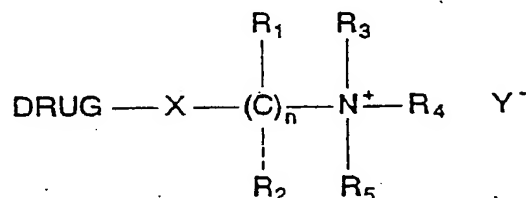
DETAILED DESCRIPTION OF THE INVENTION

20 The present invention provides novel pharmaceutical compositions that act as steroid agonists or antagonists or have mixed agonist-antagonist activity with the limitation that these
25 molecules have a permanent ionic moiety that prevents their penetration into the CNS. Contrary to the teachings of the prior art, these permanently charged derivatives of drugs can achieve their desired
30 therapeutic activity in target cells even when the receptors may be located intracellularly.

 Accordingly, the present invention provides novel pharmaceutical compositions that retain antiestrogenic activity in tumor tissue while being largely incapable of penetrating into the brain. More specifically
35 compounds according to the present invention provide

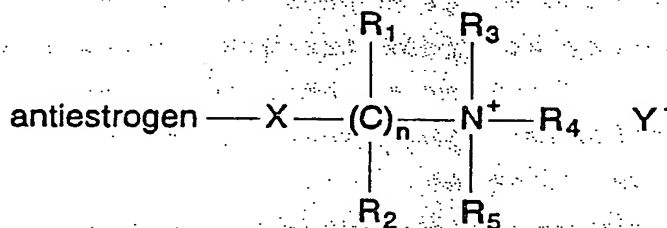
anti-tumor activity in the breast or other reproductive organs and additionally provide partial estrogenic activity in organs such as bone or the cardiovascular system where estrogen activity is beneficial. A most preferred embodiment of the present invention is devoid of CNS activity, due to its inability to cross the BBB, while simultaneously being efficacious as an anti-tumor agent (irrespective of the mechanisms involved) and as an estrogenic agent in non-tumor tissues.

One aspect of the present invention is pharmaceutical compositions that have anti-tumor activity and that contain as an active ingredient a therapeutically effective quantity of a compound of the formula



wherein Y⁻ is any non-toxic pharmaceutically acceptable anion, DRUG is a steroid agonist or antagonist, a mixed agonist-antagonist, or a partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 1-12 or is 0, in which case DRUG or the X moiety is directly attached to the quaternary nitrogen atom.

A preferred subgeneric grouping of said pharmaceutical compositions contains as an active ingredient a therapeutically effective quantity of a compound of the formula



wherein antiestrogen is an estrogen antagonist, mixed agonist-antagonist, or partial agonist and the remaining variables are as defined above.

As discussed in more detail hereinbelow, said pharmaceutical compositions will ordinarily contain a pharmaceutically acceptable diluent or carrier, for instance: a diluent comprising an aqueous cosolvent solution comprising a pharmaceutically acceptable cosolvent, a micellar solution prepared with natural or synthetic ionic or nonionic surfactants, or a combination of such cosolvent and micellar solutions; a diluent consisting essentially of a solution of ethanol, a surfactant, and water; a diluent consisting essentially of an emulsion comprising triglycerides, lecithin, glycerol, an emulsifier, an antioxidant, and water; or a carrier selected from the group consisting of corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, and gums.

Said pharmaceutical compositions can be formulated as a tablet for oral dosage, or be otherwise prepared in unit dosage form. A typical daily dosage of said compound will be from about 0.01

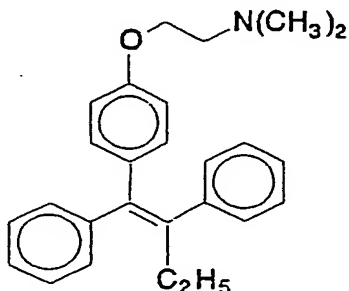
to about 10 mg/kg body weight, more preferably, from about 0.05 to about 5 mg/kg body weight.

Also contemplated according to the present invention are methods such as a method of treatment of tumors which comprises administering to a patient a therapeutically effective amount of a pharmaceutical composition as defined above. Thus the present compositions may be used for treating cancer of the breast, ovaries, uterus, or prostate, and for preventing or retarding the growth of cancer, malignant cells, or neoplasms, and for reducing or preventing the metastasis of cancer-cells.

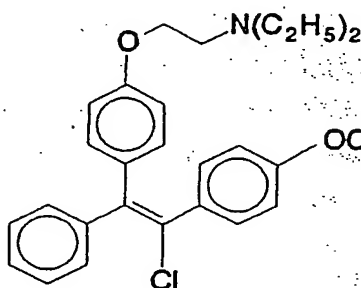
The antiestrogens

Various classes of antiestrogens can be modified in accord with the precepts of the present invention. These include: (a) antiestrogens derived from triphenylethylene, such as tamoxifen, toremifene and clomiphene; (b) antiestrogens derived from diphenyl naphthalene, such as nafoxidine; and (c) antiestrogens derived from triphenyl ethanol, such as ethamoxytriphetol.

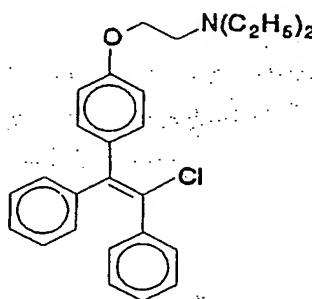
Tamoxifen, which has the following formula, may be regarded as a triphenylethylenic antiestrogen.



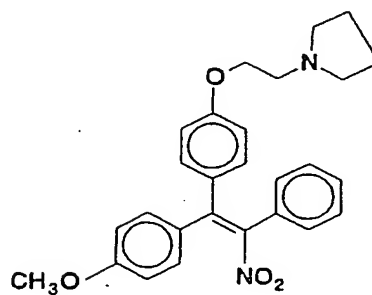
Other triphenylethylenic antiestrogens include
enclomiphene,



10
zuclophene,



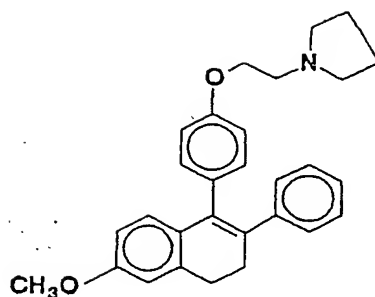
20
nitromifene,



30
nafoxidene,

35

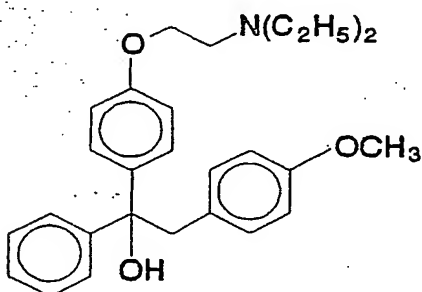
5



10 desmethyltamoxifen, toremifene, and desmethyl-toremifene.

Structurally similar antiestrogens include ethamoxypiphetol,

15

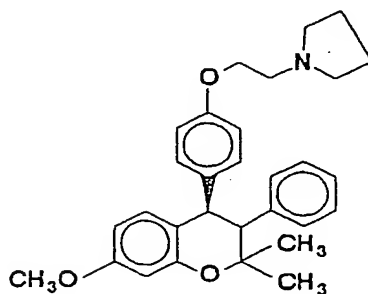


20

centchroman,

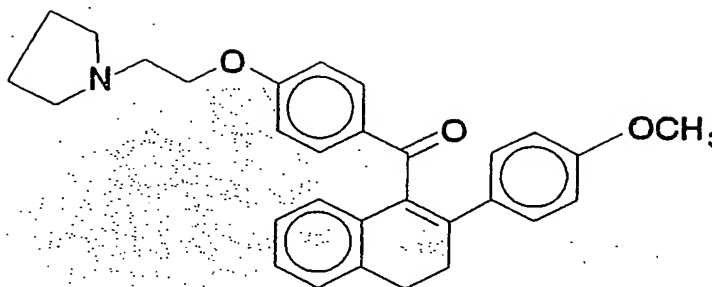
25

30



and trioxifene

35



5

10 The permanent ionic charge

The modification of drugs to prevent their access to the CNS may be most conveniently accomplished by preparation of quaternary salts. Such compounds may be prepared by a variety of chemical reactions. In the case of antiestrogens containing an amino nitrogen side chain, such as tamoxifen and its analogues, one such method is to react the antiestrogen with an alkylating agent, in order to quaternize the nitrogen atom on the side chain. The alkylating agent can be an alkyl halide, tosylate, alkyl or dialkyl sulfate or any other appropriate moiety. The alkylation may be performed with or without addition of organic solvents, as appropriate, and may be carried out under cooling or at room temperature or with heating, as appropriate, to ensure that the reaction proceeds satisfactorily to completion. However, cooling is preferable whenever cis-trans isomerization is possible. The reaction may be monitored by standard analytical methods known to one skilled in the art including thin layer chromatography, high pressure liquid chromatography, nuclear magnetic resonance spectroscopy or any other suitable method. The resulting quaternary salt is purified by standard methods, known to the artisan, usually including at least one step involving recrystallization. The

15
20
25
30
35

associated anion may be changed if desired by standard procedures such as ion-exchange columns.

Pharmaceutically acceptable anions in accordance with the present invention include citrates, chlorides, bromides, iodides, tosylates, mesylates, sulfates, and in general any anions derivable from alkylating agents or analogues thereof and which are nontoxic.

Pharmacology

10 The compound provided can be formulated by any required method to provide pharmaceutical compositions suitable for administration to a patient.

15 The novel compositions contain, in addition to the active ingredient, conventional pharmaceutically acceptable carriers, diluents and the like. Solid compositions for oral administration, such as tablets, pills, capsules or the like, may be prepared by mixing the active ingredient with conventional, pharmaceutically acceptable ingredients such as corn
20 starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate and gums, with pharmaceutically acceptable diluents. The tablets or pills can be coated or otherwise compounded with pharmaceutically acceptable materials known in
25 the art to provide a dosage form affording prolonged action or sustained release. Other solid compositions can be prepared as microcapsules for parenteral administration. Liquid forms may be prepared for oral administration or for injection, the term including
30 subcutaneous, intramuscular, intravenous, and other parenteral routes of administration. The liquid compositions include aqueous solutions, with or without organic cosolvents, aqueous or oil suspensions, emulsions with edible oils, as well as
35 similar pharmaceutical vehicles. In addition, the

compositions of the present invention may be formed as encapsulated pellets or other depots, for sustained delivery.

5 The active dose for humans is generally in the range of from 0.01 mg to about 10 mg per kg body weight, in a regimen of 1-4 times a day. However, administration at longer intervals may also be possible, for compounds or formulations having prolonged action. The preferred range of dosage is 10 from 0.05 to 5 mg per kg body weight. It is evident to one skilled in the art that dosage form and regimen would be determined by the attending physician, according to the disease to be treated, method of administration, and the patient's general condition. 15 It will be appreciated that the most appropriate administration of the pharmaceutical compositions of the present invention will depend first and foremost on the clinical indication being treated. The prophylactic treatment of healthy women at high risk 20 for malignant breast tumors will necessitate a sustained maintenance dosage regimen. In contradistinction, the treatment of existing breast cancer will require higher doses at more frequent intervals.

25

Biological activity

The present invention provides novel medical uses for both known compounds (Jarman et al., *Anticancer Drug Design*, 1986, 1, 259) and the novel antiestrogen 30 derivatives as described above. These compounds have unexpectedly been shown to possess improved anti-tumor activity when compared to tamoxifen. In addition, these compounds exhibit peripheral estrogenic and antiestrogenic activity in vivo, without appearing to 35 influence the brain. Estrogenic, antiestrogenic, and

anti-tumor activity are demonstrated in the Examples hereinbelow.

It should be noted that in those instances where tumor metastases in the CNS may be suspected, the use of non-BBB penetrating compounds of the present invention should not be contraindicated, since tumors in the CNS cause local disruption of the BBB. Indeed, in cases of metastatic brain tumors the use of quaternized anti-tumor agents may achieve preferential drug delivery to the tumor due to the known disruption of the BBB at the tumor site.

EXAMPLES

In order to further illustrate the present invention, specific examples are given below. It is to be understood that the examples given are for illustration only and are in no way limiting.

EXAMPLE 1

Tamoxifen (2.0 g) and methyl iodide (13 ml) were mixed together and the mixture held at 0°C for 24 hrs. Ethyl acetate (15 ml) was added and the white solid collected by filtration, rinsed with ethyl acetate and dried to afford 2.7 grams of tamoxifen N-methyl iodide (tamoxifen methiodide). All operations were performed at 0-5°C to avoid cis-trans isomerization.

EXAMPLE 2

A mixture of tamoxifen (0.5 g) and ethyl iodide (1.5 ml) was stored for 24 hrs at 0-5°C. Ethyl acetate was added and the solid collected by filtration and rinsed. After drying under vacuum, 0.7

g of tamoxifen N-ethyl iodide were obtained. All operations were conducted at 0-5°C.

EXAMPLE 3

5 Analogous to Example 2, tamoxifen (0.5 g), propyl iodide (1.5 ml) and ether (3 ml) were reacted for one week at 0-5°C and the resulting white solid was collected by filtration to yield 0.41 g of tamoxifen N-propyl iodide.

10

EXAMPLE 4

Tamoxifen (0.5 g) and bromomethane (0.25 g) in ether (5 ml) were mixed and held at 0-5°C for 24 hours until the initial sticky precipitate converted to a
15 white solid. The white solid was collected by filtration and rinsed with ether. After drying, 0.60 g of tamoxifen N-methyl bromide were obtained.

EXAMPLE 5

20 Effect of tamoxifen or tamoxifen methiodide on uterine weight in immature female rats: induction and blockade of uterine hypertrophy. Prepubertal female rats respond to treatment by estrogen and estrogen agonists by increase in uterine volume and weight.
25 Antiestrogens are known to block this effect when administered in conjunction with the agonist. Induction of uterine hypertrophy in this model is considered a standard test for *in vivo* estrogenic activity. Blockade of the estrogen induced response
30 is considered a standard test for antiestrogenic activity *in vivo*.

Groups of female rats, 22-23 days old, were injected i.p. with 25%/75% ethanol/water (vehicle), tamoxifen (0.5 mg/animal) in 25%/75% ethanol/water
35 suspension, or tamoxifen methiodide (0.4 mg/animal) in

25%/75% ethanol/water suspension. The volume injected was 150 microL. The animals were sacrificed 24 h later and the wet weight of excised uteri determined.

The results of the experiment are presented in Figure 1. The results were subjected to one-way analysis of variance, and the results are indicated with standard error bars. Tamoxifen ("TAM") and tamoxifen methiodide ("TAM-Q") significantly increased uterine weight. (Overall treatment effect: $P < 0.0006$, post-hoc analysis by Scheffe's test: tamoxifen vs. control $p < 0.015$, tamoxifen methiodide vs. control $p < 0.0008$).

EXAMPLE 6

In vivo estrogenic and anti-estrogenic activity of tamoxifen methiodide on bone and uterus: induction of creatine kinase activity. Estrogens are known to increase the activity of the enzyme creatine kinase (CK) in a number of target organs, including bone and uterus. Antiestrogens inhibit the estrogen-induced increase in CK activity. Compounds increasing bone CK activity are predicted to preserve bone mass, i.e. have estrogen-like anti-osteoporosis effects.

Groups of female rats, 22-23 days old, were injected i.p. with vehicle alone (HPCD), tamoxifen or tamoxifen methiodide at doses ranging from 0.1 to 1.0 $\mu\text{mole/animal}$, estrogen alone (0.01 $\mu\text{mole/animal}$) or a combination of estrogen with either tamoxifen or tamoxifen methiodide.

Animals were sacrificed 24 h later. Tibia and femur were excised and the epiphyses and diaphyses of the long bones collected and washed thoroughly in cold saline. Tissues were homogenized and centrifuged at 12,000xg for 5 min. The supernatant following this centrifugation was used to assay CK activity (Somjen

et al., Biochem., 1991, 277: 863). Enzyme activity was measured on a spectrophotometer at a wavelength of 350 nm. Protein was determined by the Coomassie blue method so that results could be expressed in units as CK specific activity in $\mu\text{mol}/\text{min}/\text{mg}$ protein.

Tamoxifen methiodide showed estrogenic activity comparable to that of tamoxifen in the bone (Figure 2). The anti-estrogenic potency of the two drugs in the bone was also similar. However, in the uterus tamoxifen methiodide was significantly less potent than tamoxifen as an agonist. At the doses administered, tamoxifen itself was capable of eliciting the full response achievable with estrogen, while the effect of tamoxifen methiodide was significantly lower when compared to either estrogen or tamoxifen and did not differ significantly from zero. The anti-estrogenic activity of tamoxifen methiodide in the uterus was similar to that of tamoxifen (Figure 2). These results indicate that quaternization of tamoxifen did not change its beneficial activity in the bone. Surprisingly, agonistic activity of tamoxifen methiodide in the uterus, which may lead to adverse effects including hypertrophy and endometrial cancer, was significantly reduced.

EXAMPLE 7

Effects of tamoxifen methiodide on body weight changes: induction and blockade of changes in body weight in ovariectomized female rats. Body weight in female rats is tightly regulated by estrogen, which inhibits food and water intake through direct influence on the CNS thereby limiting growth. Ovariectomy results in a cumulative increase in food and water intake and body weight. Estrogen and its

agonists prevent this effect of ovariectomy and antiestrogens reverse the outcome of estrogen. Thus, changes in body weight in ovariectomized females are a standard test for estrogenic and antiestrogenic activity. Compounds that do not cross the BBB are not expected to be effective in this model.

Pellets containing either 5 mg tamoxifen or 7 mg tamoxifen methiodide were manufactured by Innovative Research of America (Toledo, OH). Pellets containing 100 μ g of estradiol-17 β were also supplied by the same manufacturer. This study was designed to continue the evaluation of the antiestrogen effects of TAM and TAM-Q both in the central and peripheral compartments of the rat. Female Sprague-Dawley rats weighing 150-170 g were bilaterally ovariectomized and allowed a 9 day recovery period for the rat to become maximally sensitized to changes in the estrogen environment. Rats were administered either TAM, TAM-Q, estradiol or combinations of the antiestrogen with estrogen (6 animals per composition). All pellets were designed for release of the active compound over 21 days. The experimental groups are presented in Table 1.

Table 1

Animal Group No.	Treatment		Dose mg Drug/Animal
	Surgery	Therapy	
1	Ovx	TAM	15
2	Ovx	TAM-Q	21
3	Ovx	TAM/Estradiol	15/0.2
4	Ovx	TAM-Q/Estradiol	15/0.2
5	Ovx	Estradiol	0.2
6	Ovx	Placebo	--
Intact	Intact	Placebo	--

The *in vivo* phase of the study is presented in Figure 3. As can be seen from the results, TAM-Q, unlike TAM, does not block the central effects of estrogen on body weight in these animals, thus indicating that it is not capable of penetrating into the CNS. In contradistinction, as shown in Figure 1, the quaternized compound (TAM-Q) is efficient in blocking the peripheral effect of estrogen on uterine weight confirming its peripheral antiestrogen effect.

10

EXAMPLE 8

Brain and plasma levels of tamoxifen methiodide. Adult rats, 4 per group, were injected with tamoxifen methiodide, 0.5 mg/kg or an equimolar dose of tamoxifen (0.36 mg/kg) i.v. in DMSO. Fifteen minutes later, animals were sacrificed and their sera and brains were collected, homogenized and analyzed by HPLC.

The results (Table 2) show fast accumulation of tamoxifen in the brain and very low serum levels at this time point, probably due to sequestration into lipid compartments and elimination. Tamoxifen methiodide, on the other hand, was present in serum at concentrations of 1-2 $\mu\text{g/ml}$ while brain levels were below detection. These results indicate that the quaternization of tamoxifen did, indeed, render it incapable of penetration into the brain.

Table 2

30

	Serum	Brain
Tamoxifen	not detectable below 100 nanog/ml	680 \pm 49 nanog/g
Tamoxifen methiodide	1625 \pm 110 nanog/ml	not detectable below 100 nanog/ml

35

EXAMPLE 9

Anti-tumor activity of tamoxifen methiodide in mice: anti-tumor activity in nude mice implanted with human breast cancer cells. Human breast cancer cells can be grown in culture. Injection of such cells into the flank of genetically athymic nude mice, coupled with estrogen treatment, results in the emergence of a tumor within a few weeks. If left untreated, the tumors will grow over time even after withdrawal of the exogenous estrogen until death of the animals occurs. Tumor size is relatively easy to measure and the ability of compounds to halt, or most preferably to induce regression of tumor growth, is considered a test of their anti-tumor activity.

MCF7 cells (human breast cancer derived cell line) were grown in culture. A total of 30 nude mice were each injected with 2×10^6 tumor cells in the flank area. Concomitantly, a subcutaneous estrogen pellet was implanted in the back of the neck. Animals were observed for tumor emergence. When visible tumors were detected (2-4 weeks after implantation), their length, width and height were measured with calipers. The values recorded were multiplied to produce a volume measurement and noted as baseline (time 0) tumor size. At this point, the estrogen pellet was replaced by a blank pellet (9 animals/control group), a tamoxifen methiodide pellet containing a molar equivalent of 5 mg tamoxifen (10 animals), or a tamoxifen 5 mg pellet (9 animals). The pellets were purchased from Innovation Research and were designed to provide 21 days of constant release. Animals were then monitored for tumor growth and general appearance at least once a week over the next six weeks. The changes in tumor size compared to time 0 were calculated as percent change as follows:

$$\frac{(\text{tumor size at time } t - \text{tumor size at time } 0)}{(\text{tumor size at time } 0)} \times 100$$

Thus, total regression of the tumor will translate into a -100% change. Statistical analysis of variation with repeated measure revealed highly significant effects of treatment ($p < 0.0001$) and time (repeated measure, $p < 0.0001$) and a significant interaction ($p < 0.0001$). Post-hoc analysis was performed using the Scheffe F test with alpha preset to 0.05.

The results of the experiment are summarized in Figure 4, in which the symbol \odot indicates the results obtained with blank pellets, the symbol X indicates the results obtained with tamoxifen pellets, and the symbol Δ indicates the results obtained with tamoxifen methiodide pellets. As can be seen therefrom, tamoxifen methiodide unexpectedly induced significant tumor regression as early as 10 days after implantation of the pellet.

The effect of tamoxifen methiodide on tumor growth is dose dependent. In another experiment, animals treated as described above were administered daily with 2, 5 or 10 mg/kg tamoxifen methiodide in HPCD intraperitoneally, while the control group received vehicle. The low dose was not effective, the 5mg/kg dose resulted in significant reduction in tumor growth rate (Figure 5) and the high dose completely halted tumor growth by day 10 and caused significant tumor regression at later time points, similar to the results with the single dose pellet described above.

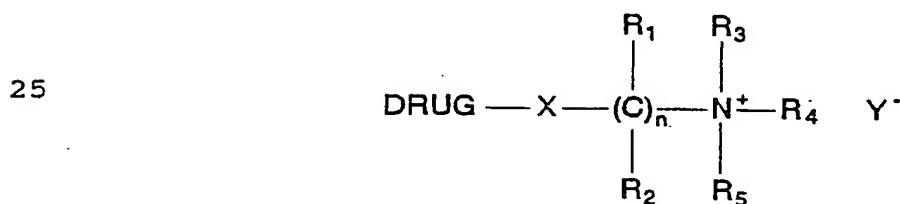
Anti-tumor activity in nude mice implanted with RAS sarcoma cells (transformed rat fibroblasts): Since it is known that tamoxifen possesses antitumor activity in estrogen-receptor negative tumors and even in tumors unrelated to reproductive or steroid target

organs, we examined the in vivo activity of tamoxifen methiodide in one such tumor. Ten male BALB/c nude mice were injected with human Ha-ras transformed rat 1 fibroblasts (2×10^6 cells/animal injected s.c. above the left femoral joint). When the tumor reached a volume of 0.5ml or more (within 7-10 days) animals were given either tamoxifen methiodide (2mg/kg/day i.p) or vehicle. By day 11 of treatment, tamoxifen methiodide resulted in a significant ($p < 0.05$) reduction of tumor growth rate which amounted to 50%. These results show that TAM-Q can inhibit the growth of ras sarcoma in vivo; so that the antitumor activity of the drug is not limited to estrogen dependent tumors.

15 NOVEL COMPOUNDS

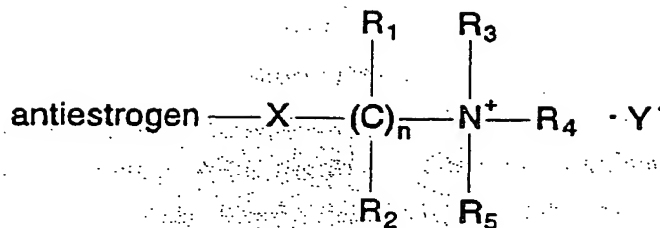
As indicated hereinabove, certain of the compounds that can be used in the pharmaceutical compositions and therapeutic methods according to the present invention are known. The following novel compounds, however, constitute in themselves another aspect of the invention herein described.

Permanently ionic compounds having the formulae



30

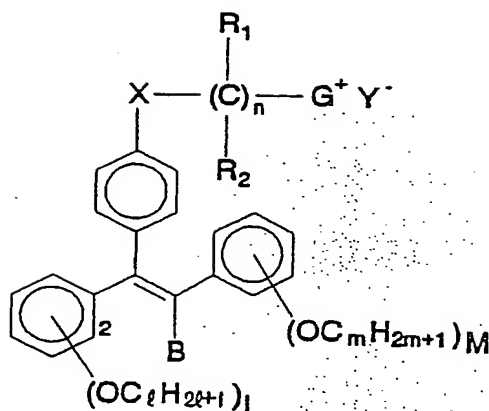
35



10 wherein Y is any non-toxic pharmaceutically acceptable
 anion, DRUG is a steroid agonist or antagonist, mixed
 agonist-antagonist, or partial agonist; antiestrogen
 is an estrogen antagonist, mixed agonist-antagonist,
 15 or partial agonist; X is a direct bond or -O-, NR₆, -S-
 , -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H,
 alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl;
 R₃, R₄, R₅ are independently branched or unbranched,
 cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up
 20 to 10 carbons atoms substituted by carboxy, hydroxy,
 alkoxy, halo, or nitro, branched or unbranched, cyclic
 or noncyclic arylalkyl of 7-16 carbons, aryl; n is 1-
 12 or 0, provided that when DRUG or antiestrogen is
 tamoxifen or 4-hydroxy-2-methyltamoxifen, R₃ and R₄ are
 25 methyl, and R₅ is methyl or ethyl, Y is not a halide.

Preferred compounds according to the above
 formulae are those wherein antiestrogen is selected
 from the group consisting of tamoxifen,
 desmethyltamoxifen, toremifene, desmethyltoremifene,
 30 clomiphene, nafoxidine, and ethamoxytriphetol.

Another grouping of novel compounds according to
 the present invention is those compounds having the
 formula



- 15 wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-,
 -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H,
 alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or
 aryl; n is 0-12; G is a moiety selected from the group
 consisting of -N(R')(R'')(R'''), -(O)N(R')(R''),
 20 -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is
 alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons
 atoms substituted by carboxy, hydroxy, alkoxy, halo,
 or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-
 alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons
 25 atoms and R'' and R''' are independently C₁-C, alkyl
 and R'' and R''' together with N may form a 4- to 8-
 membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety
 which is linked to the 2-position of the phenyl that
 is neither the phenyl linked to the same ethylene
 30 carbon as B nor the phenyl substituted by the radical
 containing the permanently ionic group G, said moiety
 being selected from the group consisting of
 -CH₂C(R₁)(R₂)- and -CH₂O-; L and M are independently
 0-3; l, m, and p are independently 1-7; and Y is a
 35 pharmaceutically acceptable anion, provided that when

G is $-N(R')(R'')(R''')$ or $-(O)N(R')(R'')$, R' and R'' cannot both be unsubstituted alkyl.

Preferred compounds of this grouping are those wherein X is $-O-$; R_1 and R_2 are H; n is 2; G is
5 $-N(R')(R'')(R''')$; B is CH_3 , C_2H_5 , halo, nitro, or a moiety which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the permanently ionic group G, said moiety
10 being selected from the group consisting of $-CH_2CH_2-$ and $-CH_2O-$; L and M are 0 or 1, l and m are 1, and p is 2; and Y is a pharmaceutically acceptable anion.

These compounds have utility as peripheral antiestrogens effective in the clinical treatment of
15 cancer and other diseases and pathological conditions. These peripheral antiestrogens will possess estrogen antagonist activity, and may possess partial estrogen agonist or mixed activity. The compounds, however, are limited in biodistribution by being excluded from
20 the CNS and brain, thereby exhibiting reduced side effects. It is this limited biodistribution that significantly enhances the clinical usefulness of the present compounds. Another useful aspect of the compounds of this invention that enhances their
25 attractiveness for clinical use is the comparatively elevated circulatory levels of these agents due to the fact that they are not sequestered in fat tissue, thereby allowing for more precise control of dosing. The use of the present compounds in the treatment of
30 cancerous tumors has been demonstrated above.

While the invention has been described in terms of various preferred embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions and changes may be made
35 without departing from the spirit thereof.

Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims.

5

10

15

20

25

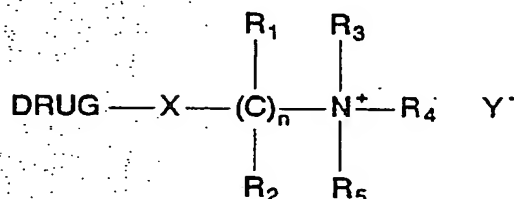
30

35

THE CLAIMS

What is claimed is:

- 5 1. A pharmaceutical composition having bioaffecting activity selected from the group consisting of anti-inflammatory activity and anti-tumor activity which contains as an active ingredient a therapeutically effective quantity of a compound of
10 the formula

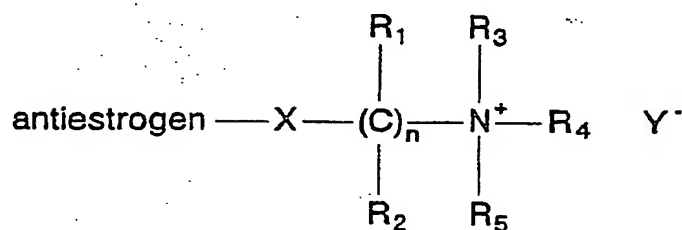


15 wherein Y is any non-toxic pharmaceutically acceptable anion, DRUG is a steroid agonist or antagonist, mixed
20 agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently
25 branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12.

30

35

2. A pharmaceutical composition having anti-tumor activity which contains as an active ingredient a therapeutically effective quantity of a compound of the formula



wherein Y⁻ is any non-toxic pharmaceutically acceptable anion, antiestrogen is an estrogen antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12.

3. A pharmaceutical composition according to claim 1 further containing a pharmaceutically acceptable diluent or carrier.

4. A pharmaceutical composition according to claim 3 wherein the diluent comprises an aqueous cosolvent solution of a pharmaceutically acceptable cosolvent, a micellar solution prepared with natural or synthetic ionic or nonionic surfactants, or a combination of such cosolvent and micellar solutions.

5. A pharmaceutical composition according to claim 3 wherein the diluent consists essentially of a solution of ethanol, a surfactant, and water.

5 6. A pharmaceutical composition according to claim 3 wherein the diluent consists essentially of an emulsion comprising triglycerides, lecithin, glycerol, an emulsifier, an antioxidant, and water.

10 7. A pharmaceutical composition according to claim 3 wherein the carrier is selected from the group consisting of corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, and gums.

15 8. A pharmaceutical composition according to claim 7 formulated as a tablet for oral dosage.

20 9. A pharmaceutical composition according to claim 3 in unit dosage form.

10. A pharmaceutical composition according to claim 1 wherein the daily dosage of said compound from about 0.01 to about 10 mg/kg body weight.

25 11. A pharmaceutical composition according to claim 10 wherein said daily dosage is from about 0.05 to about 5 mg/kg body weight.

30 12. A pharmaceutical composition according to claim 2 wherein antiestrogen is tamoxifen.

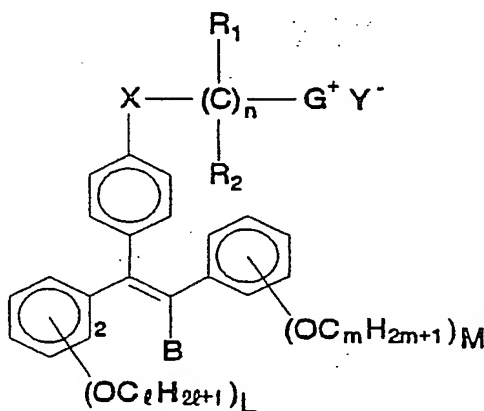
13. A pharmaceutical composition according to claim 2 wherein antiestrogen is toremifene.

35

14. A pharmaceutical composition according to claim 2 wherein antiestrogen is clomifene.

15. A pharmaceutical composition according to claim 2 wherein antiestrogen is nafoxidine.

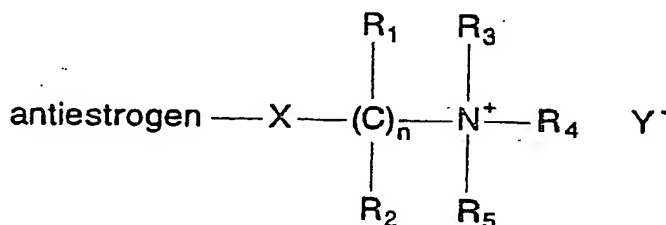
16. A pharmaceutical composition having anti-tumor activity which contains as an active ingredient a therapeutically effective quantity of a compound of the formula



wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H, alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety

which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the permanently ionic group G, said moiety
 5 being selected from the group consisting of
 $-\text{CH}_2\text{C}(\text{R}_1)(\text{R}_2)-$ and $-\text{CH}_2-\text{O}-$; L and M are independently 0-3; l, m, and p are independently 1-7; and Y is a pharmaceutically acceptable anion.

10 17. A permanently ionic compound having the formula



20 wherein Y⁻ is any non-toxic pharmaceutically acceptable anion, antiestrogen is an estrogen antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of
 25 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or
 30 nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12, provided that when antiestrogen is tamoxifen or 4-hydroxy-2-methyltamoxifen, R₃ and R₄ are methyl, and R₅ is methyl or ethyl, Y is not a halide.

18. A permanently ionic compound as in claim 17 having the formula:



10 wherein Y is any non-toxic pharmaceutically acceptable anion; antiestrogen-N-(A)₂ is an antiestrogen, estrogen antagonist, estrogen mixed agonist-antagonist, or partial agonist; R is a branched or unbranched, cyclic or non-cyclic alkyl, arylalkyl, or aryl hydrocarbyl group of 1-16 carbons; and X is selected from the group consisting of citrates, chlorides, bromides, iodides, tosylates, mesylates, and sulfates.

20 19. A permanently ionic compound as in claim 18 wherein antiestrogen is selected from the group consisting of tamoxifen, desmethyldtamoxifen, toremifene, desmethyldtoremifene, clomiphene, nafoxidine, and ethamoxyltriphetol.

25

20. A permanently ionic compound according to claim 18 wherein antiestrogen is tamoxifen.

30 21. A permanently ionic compound according to claim 18 wherein antiestrogen is toremifene.

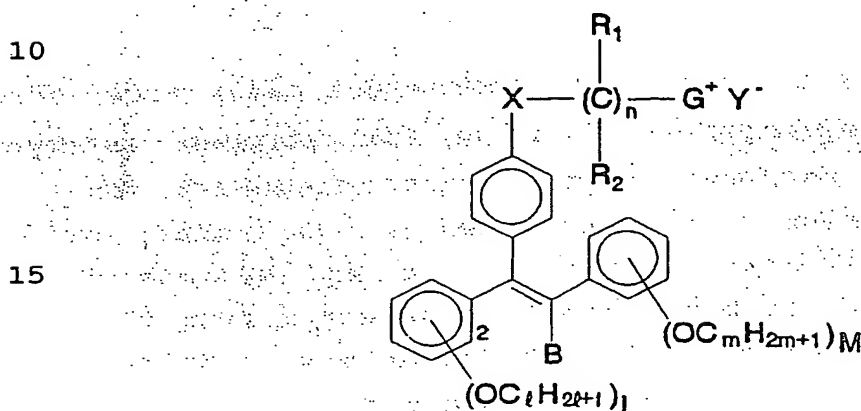
22. A permanently ionic compound according to claim 18 wherein antiestrogen is clomifene.

35

23. A permanently ionic compound according to claim 18 wherein antiestrogen is nafoxidine.

24. A quaternary salt according to claim 19 wherein R is CH_2 , X is H and Y is a pharmaceutically acceptable anion.

25. A compound of the formula

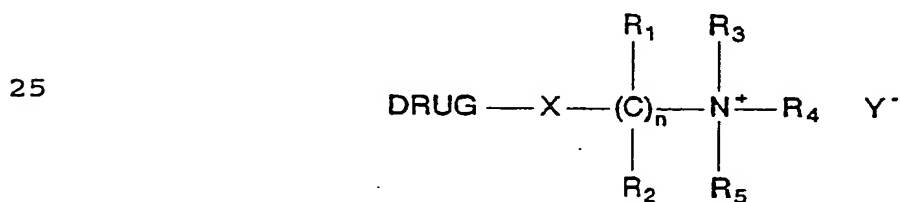


wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H, alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene

carbon as B nor the phenyl substituted by the radical containing the permanently ionic group G, said moiety being selected from the group consisting of $-\text{CH}_2\text{C}(\text{R}_1)(\text{R}_2)-$ and $-\text{CH}_2-\text{O}-$; L and M are independently 0-3; l, m, and p are independently 1-7; and Y is a pharmaceutically acceptable anion, provided that when G is $-\text{N}(\text{R}')(\text{R}'')(\text{R}''')$ or $-(\text{O})\text{N}(\text{R}')(\text{R}'')$, R' and R'' cannot both be unsubstituted alkyl.

26. A compound as in claim 25 wherein X is $-\text{O}-$; R_1 and R_2 are H; n is 2; G is $-\text{N}(\text{R}')(\text{R}'')(\text{R}''')$; B is CH_3 , C_2H_5 , halo, nitro, or a moiety which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the permanently ionic group G, said moiety being selected from the group consisting of $-\text{CH}_2\text{CH}_2-$ and $-\text{CH}_2-\text{O}-$; L and M are 0 or 1, l and m are 1, and p is 2; and Y is a pharmaceutically acceptable anion.

27. A permanently ionic compound having the formula



wherein Y^- is any non-toxic pharmaceutically acceptable anion; DRUG is a steroid antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or $-\text{O}-$, NR_6 , $-\text{S}-$, $-\text{SO}-$, $-\text{SO}_2-$, or $-\text{PO}_3-$; R_1 and R_2 are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R_3 , R_4 , R_5 are independently branched

or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12, provided that when DRUG is tamoxifen or 4-hydroxy-2-methyltamoxifen, R₃ and R₄ are methyl, and R₅ is methyl or ethyl, Y is not a halide.

28. A method for treating inflammatory conditions selected from the group consisting of acute, chronic, and recurrent inflammatory diseases and autoimmune diseases which comprises administering a therapeutically effective amount of a corticosteroid derivative according to claim 1.

29. A method for treating hormone deficiencies which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 1.

30. A method of treatment of tumors which comprises administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 1.

31. A method for treating cancer of the breast, ovaries, or prostate which comprises administering a therapeutically acceptable amount of a pharmaceutical composition according to claim 1.

32. A method according to claim 31 wherein DRUG is a synthetic antiestrogen.

33. A method according to claim 31 wherein DRUG is a synthetic progestin.

34. A method for preventing or retarding the growth of cancer, malignant cells, or neoplasms which comprises administering to the cells or organisms a therapeutically effective amount of a pharmaceutical composition according to claim 1.

35. A method for reducing or preventing the metastasis of cancer-cells which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 1.

15

20

25

30

35

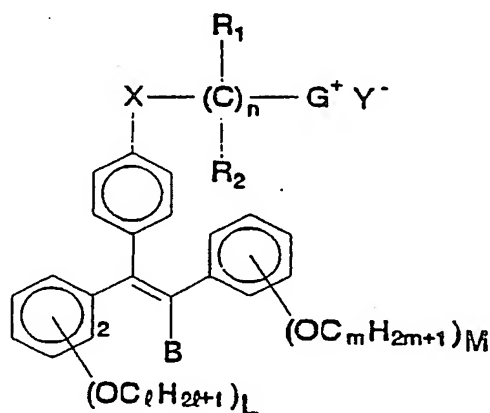
AMENDED CLAIMS

[received by the International Bureau on 05 September 1995 (05.09.95);
original claims 16-23 and 25-27 amended; new claims 36-39 added;
remaining claims unchanged (11 pages)]

14. A pharmaceutical composition according to claim 2 wherein antiestrogen is clomifene.

15. A pharmaceutical composition according to claim 2 wherein antiestrogen is nafoxidine.

16. A pharmaceutical composition having anti-tumor activity which contains as an active ingredient a therapeutically effective quantity of a compound of the formula



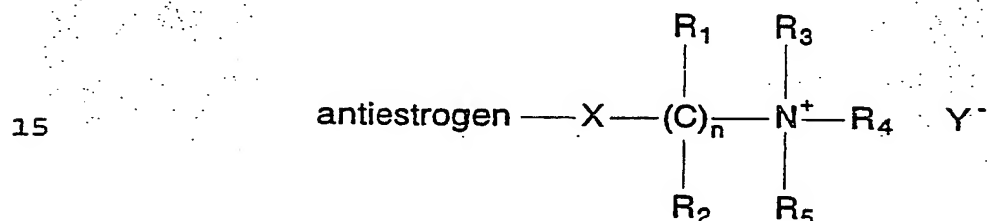
wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H, alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons atoms and R'' and R''' are independently C₁-C, alkyl and R'' and R''' together with N may form a 4- to 8-membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety

43

which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the ionic group G, said moiety being

- 5 selected from the group consisting of $-\text{CH}_2\text{C}(\text{R}_1)(\text{R}_2)-$ and $-\text{CH}_2-\text{O}-$; L and M are independently 0-3; l, m, and p are independently 1-7; and Y is a pharmaceutically acceptable anion.

- 10 17. A quaternary ammonium salt having the formula



- 20 wherein Y⁻ is any non-toxic pharmaceutically acceptable anion, antiestrogen is an estrogen antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently 25 branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic 30 arylalkyl of 7-16 carbons, aryl; n is 0-12, provided that when antiestrogen is tamoxifen or 4-hydroxy-2-methyltamoxifen, R₃ and R₄ are methyl, and R₅ is methyl or ethyl, Y is not a halide.

35

44

18. A quaternary ammonium salt having the formula:



10 wherein Y⁻ is any non-toxic pharmaceutically acceptable anion; and antiestrogen-N-(A)₂ is an antiestrogen, estrogen antagonist, estrogen mixed agonist-antagonist, or partial agonist; R is a branched or unbranched, cyclic or non-cyclic alkyl, arylalkyl, or
15 aryl hydrocarbyl group of 1-16 carbons, provided that when antiestrogen is tamoxifen or 4-hydroxy-2-methyltamoxifen, both A groups are methyl, or one A group is methyl and the other is ethyl, Y⁻ is not a halide.

20

19. A quaternary ammonium salt as in claim 18 wherein antiestrogen is selected from the group consisting of tamoxifen, desmethyltamoxifen, toremifene, desmethyltoremifene, clomiphene,
25 nafoxidine, and ethamoxytriphetol.

20. A quaternary ammonium salt according to claim 18 wherein antiestrogen is tamoxifen.

30

21. A quaternary ammonium salt according to claim 18 wherein antiestrogen is toremifene.

22. A quaternary ammonium salt according to claim 18 wherein antiestrogen is clomifene.

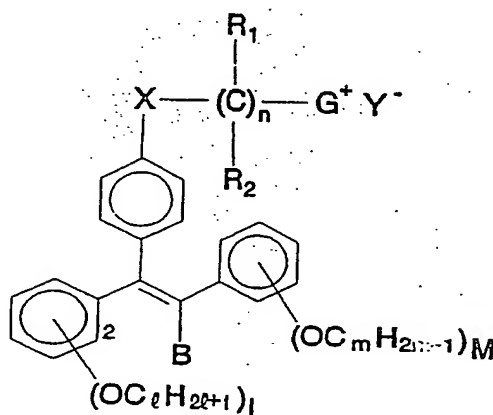
35

45

23. A quaternary ammonium salt according to claim 18 wherein antiestrogen is nafoxidine.

24. A quaternary salt according to claim 19 wherein R is CH₂, X is H and Y⁻ is a pharmaceutically acceptable anion.

25. A compound of the formula



20 wherein X is a direct bond or is -O-, -NR₆-, -S-, -SO-, -SO₂-, or -PO₃-; R₁, R₂, and R₆ are independently H, alkyl of 1-10 carbons, aralkyl of 7-16 carbons, or aryl; n is 0-12; G is a moiety selected from the group

25 consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-

30 alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbons atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring; B is C_pH_{2p+1}, halo, nitro, or a moiety which is linked to the 2-position of the phenyl that

35 is neither the phenyl linked to the same ethylene

46

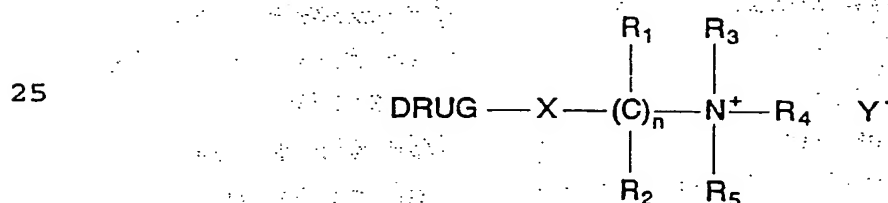
carbon as B nor the phenyl substituted by the radical containing the ionic group G, said moiety being selected from the group consisting of

-CH₂C(R₁)(R₂)- and -CH₂-O-; L and M are independently

0-3; l, m, and p are independently 1-7; and Y is a pharmaceutically acceptable anion, provided that when G is -N(R')(R'')(R''') or -(O)N(R')(R''), R' and R'' cannot both be unsubstituted alkyl.

26. A compound as in claim 25 wherein X is -O-; R₁ and R₂ are H; n is 2; G is -N(R')(R'')(R'''); B is CH₃, C₂H₅, halo, nitro, or a moiety which is linked to the 2-position of the phenyl that is neither the phenyl linked to the same ethylene carbon as B nor the phenyl substituted by the radical containing the ionic group G, said moiety being selected from the group consisting of -CH₂CH₂- and -CH₂-O-; L and M are 0 or 1, l and m are 1, and p is 2; and Y is a pharmaceutically acceptable anion.

27. A quaternary ammonium salt having the formula



wherein Y⁻ is any non-toxic pharmaceutically acceptable anion; DRUG is a steroid antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched

47

or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbons atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12, provided that when DRUG is tamoxifen or 4-hydroxy-2-methyltamoxifen, R₃ and R₄ are methyl, and R₅ is methyl or ethyl, Y is not a halide.

28. A method for treating inflammatory conditions selected from the group consisting of acute, chronic, and recurrent inflammatory diseases and autoimmune diseases which comprises administering a therapeutically effective amount of a corticosteroid derivative according to claim 1.

29. A method for treating hormone deficiencies which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 1.

30. A method of treatment of tumors which comprises administering to a patient a therapeutically effective amount of a pharmaceutical composition according to claim 1.

31. A method for treating cancer of the breast, ovaries, or prostate which comprises administering a therapeutically acceptable amount of a pharmaceutical composition according to claim 1.

32. A method according to claim 31 wherein DRUG is a synthetic antiestrogen.

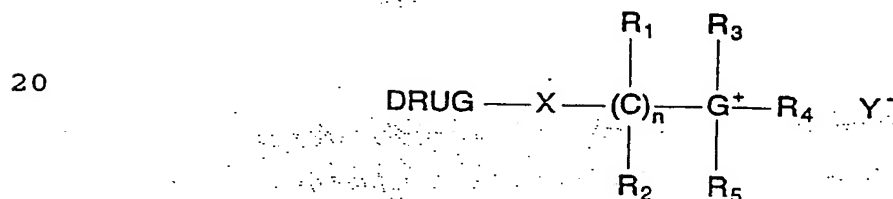
33. A method according to claim 31 wherein DRUG is a synthetic progestin.

48

34. A method for preventing or retarding the growth of cancer, malignant cells, or neoplasms which comprises administering to the cells or organisms a therapeutically effective amount of a pharmaceutical composition according to claim 1.

35. A method for reducing or preventing the metastasis of cancer-cells which comprises administering a therapeutically effective amount of a pharmaceutical composition according to claim 1.

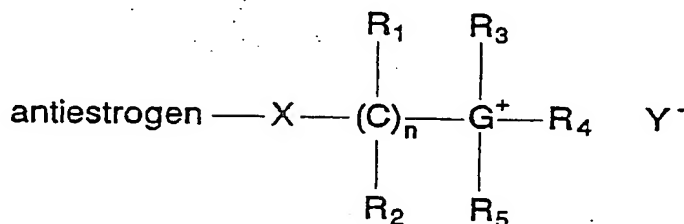
36. A pharmaceutical composition having bioaffecting activity selected from the group consisting of anti-inflammatory activity and anti-tumor activity which contains as an active ingredient a therapeutically effective quantity of a compound of the formula



wherein Y^- is any non-toxic pharmaceutically acceptable anion, DRUG is a steroid agonist or antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR_6 , -S-, -SO-, -SO₂-, or -PO₃-; R_1 and R_2 are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R_3 , R_4 , R_5 are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12; G is a

moiety selected from the group consisting of
 $-N(R')(R'')(R''')$, $-(O)N(R')(R'')$, $-S(R')(R'')$, and
 $-P(R')(R'')(R''')$, wherein R' is alkyl of 1-10 carbon
 atoms, alkyl of up to 10 carbon atoms substituted by
 5 carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl
 of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon
 atoms, or aralkyl of 7-16 carbon atoms and R'' and
 R''' are independently C_1-C_7 alkyl and R'' and R'''
 together with N may form a 4- to 8-membered ring.

10 37. A pharmaceutical composition having anti-
 tumor activity which contains as an active ingredient
 a therapeutically effective quantity of a compound of
 the formula



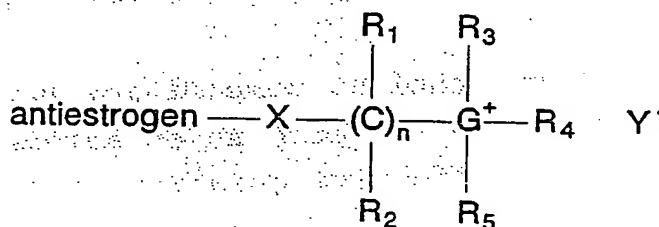
wherein Y^- is any non-toxic pharmaceutically acceptable
 anion, antiestrogen is an estrogen antagonist, mixed
 25 agonist-antagonist, or partial agonist; X is a direct
 bond or $-O-$, NR_6 , $-S-$, $-SO-$, $-SO_2-$, or $-PO_3-$; R_1 and R_2
 are independently H, alkyl of 1-10 carbon, aralkyl of
 7-16 carbons or aryl; R_3 , R_4 , R_5 are independently
 branched or unbranched, cyclic or noncyclic alkyl of
 30 1-10 carbons, alkyl of up to 10 carbon atoms
 substituted by carboxy, hydroxy, alkoxy, halo, or
 nitro, branched or unbranched, cyclic or noncyclic
 arylalkyl of 7-16 carbons, aryl; n is 0-12; G is a
 moiety selected from the group consisting of
 35 $-N(R')(R'')(R''')$, $-(O)N(R')(R'')$, $-S(R')(R'')$, and

50

-P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbon atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring.

38. An ionic compound having the formula

10



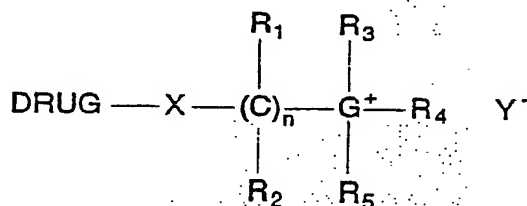
15

wherein Y⁻ is any non-toxic pharmaceutically acceptable anion, antiestrogen is an estrogen antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR₆, -S-, -SO-, -SO₂-, or -PO₃-; R₁ and R₂ are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R₃, R₄, R₅ are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), -(O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbon atoms and R'' and R''' are independently C₁-C₇ alkyl and R'' and R'''

51

together with N may form a 4- to 8-membered ring, provided that when antiestrogen is tamoxifen or 4-hydroxy-2-methyltamoxifen, R_3 and R_4 are methyl, and R_5 is methyl or ethyl, Y is not a halide.

39. An ionic compound having the formula



wherein Y^- is any non-toxic pharmaceutically acceptable anion; DRUG is a steroid antagonist, mixed agonist-antagonist, or partial agonist; X is a direct bond or -O-, NR_6 , -S-, -SO-, -SO₂-, or -PO₃-; R_1 and R_2 are independently H, alkyl of 1-10 carbon, aralkyl of 7-16 carbons or aryl; R_3 , R_4 , R_5 are independently branched or unbranched, cyclic or noncyclic alkyl of 1-10 carbons, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, branched or unbranched, cyclic or noncyclic arylalkyl of 7-16 carbons, aryl; n is 0-12; G is a moiety selected from the group consisting of -N(R')(R'')(R'''), - (O)N(R')(R''), -S(R')(R''), and -P(R')(R'')(R'''), wherein R' is alkyl of 1-10 carbon atoms, alkyl of up to 10 carbon atoms substituted by carboxy, hydroxy, alkoxy, halo, or nitro, cycloalkyl of 4-8 carbon atoms, cycloalkyl-alkyl of 5-18 carbon atoms, or aralkyl of 7-16 carbon atoms and R'' and R''' are independently C₁-C₄ alkyl and R'' and R''' together with N may form a 4- to 8-membered ring, provided that when DRUG is tamoxifen or 4-hydroxy-2-methyltamoxifen,

52

R_3 and R_4 are methyl, and R_5 is methyl or ethyl, Y is not a halide.

5

10

15

20

25

30

35

1/5

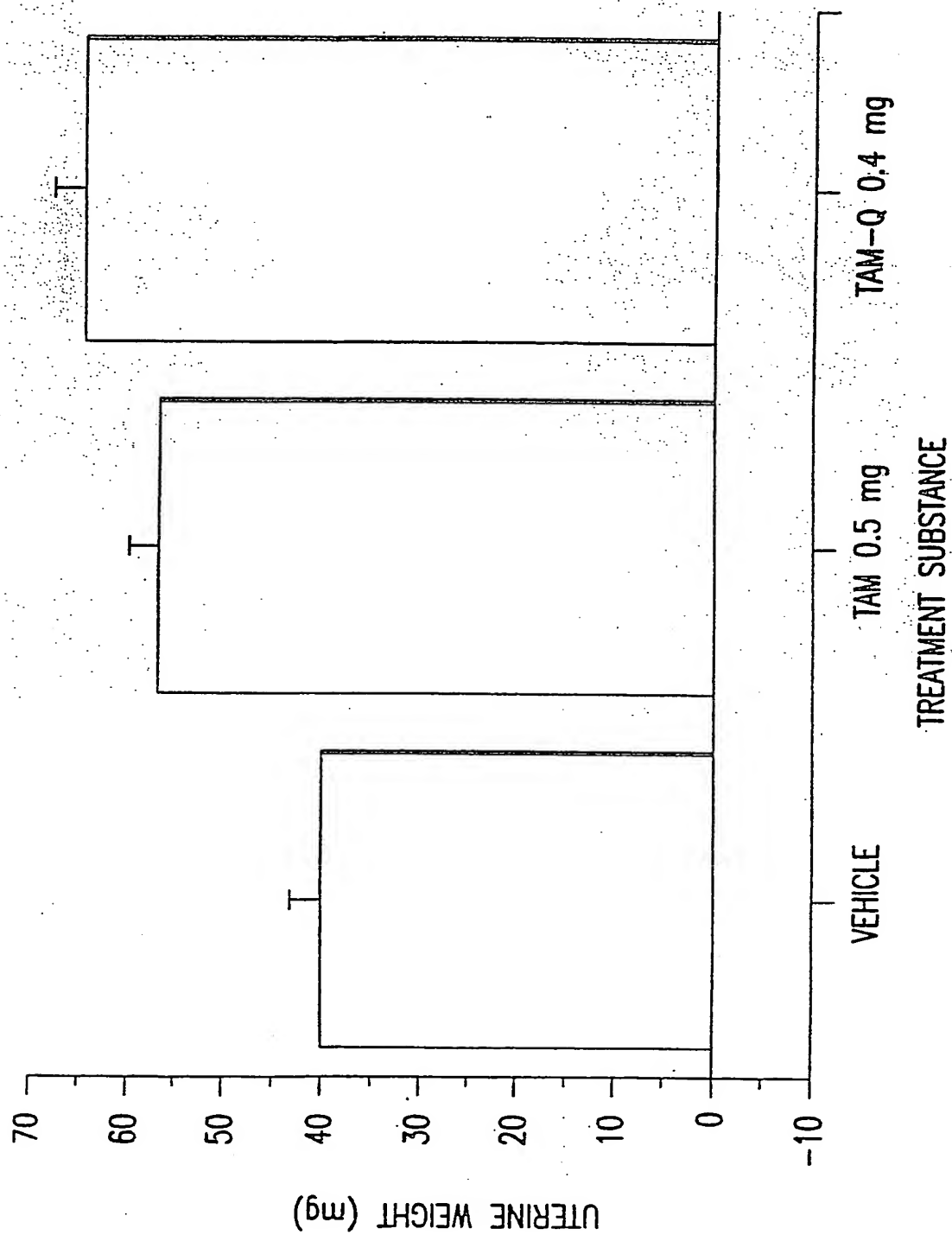


FIG. 1

2/5

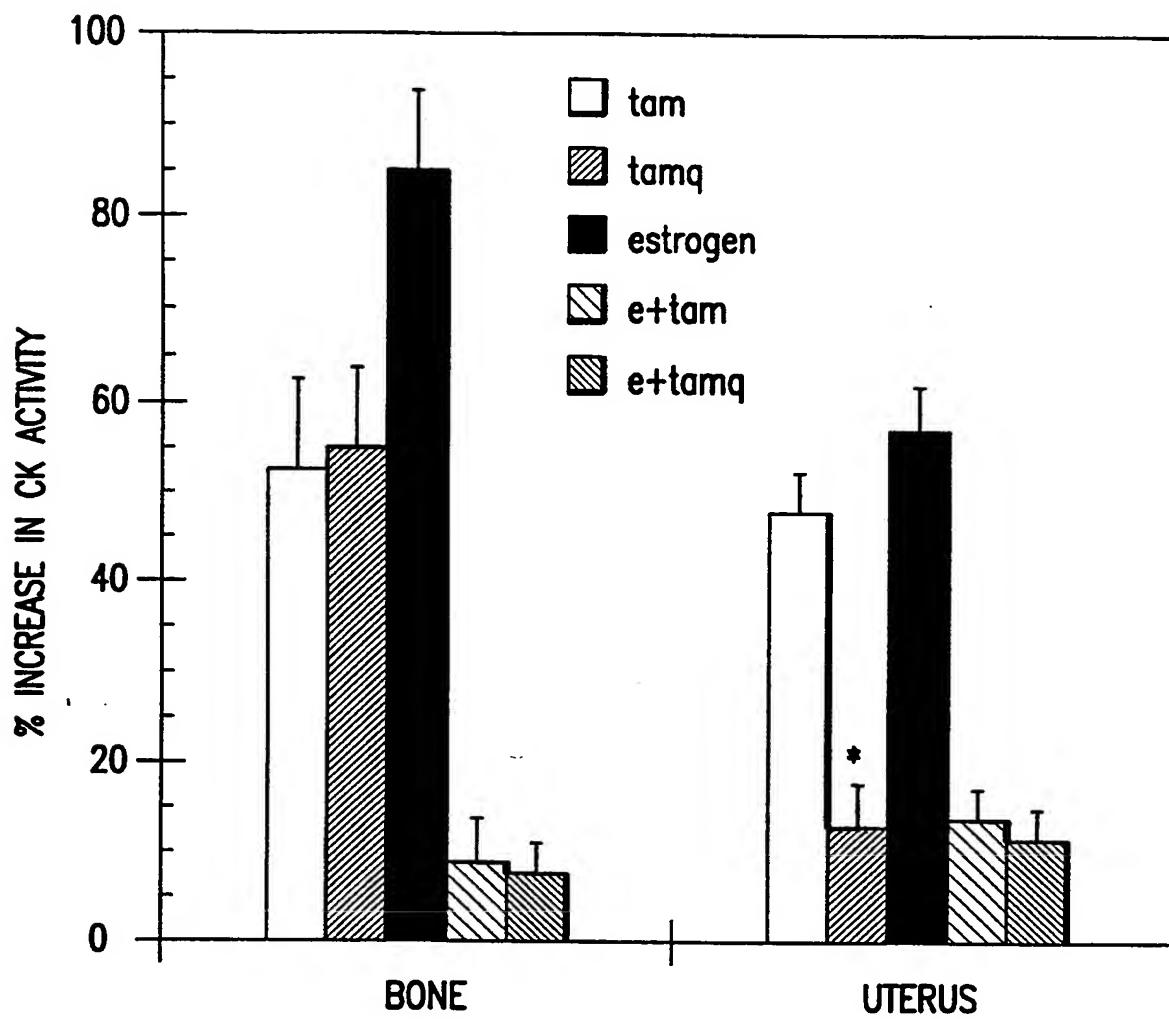


FIG.2

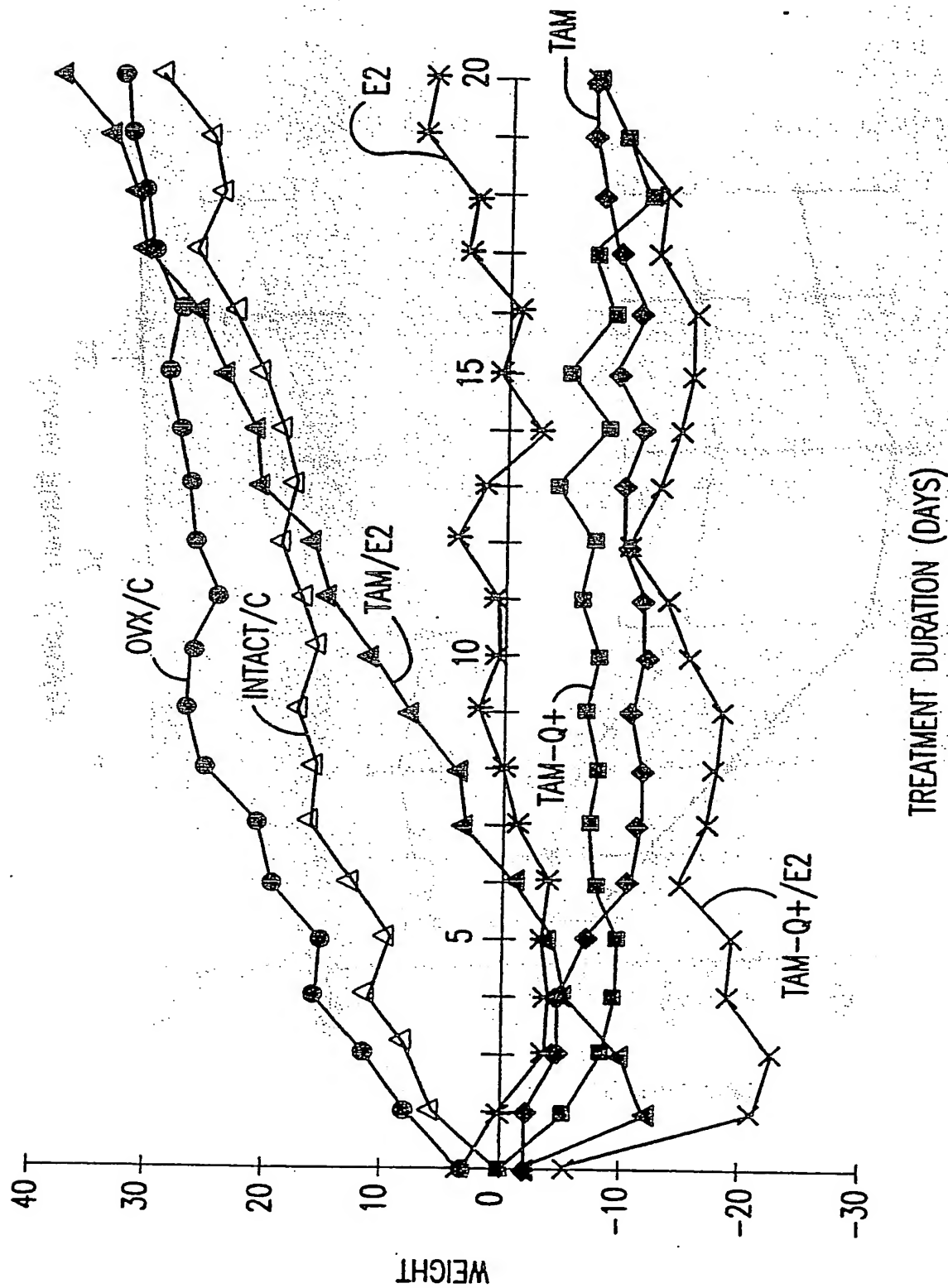


FIG. 3

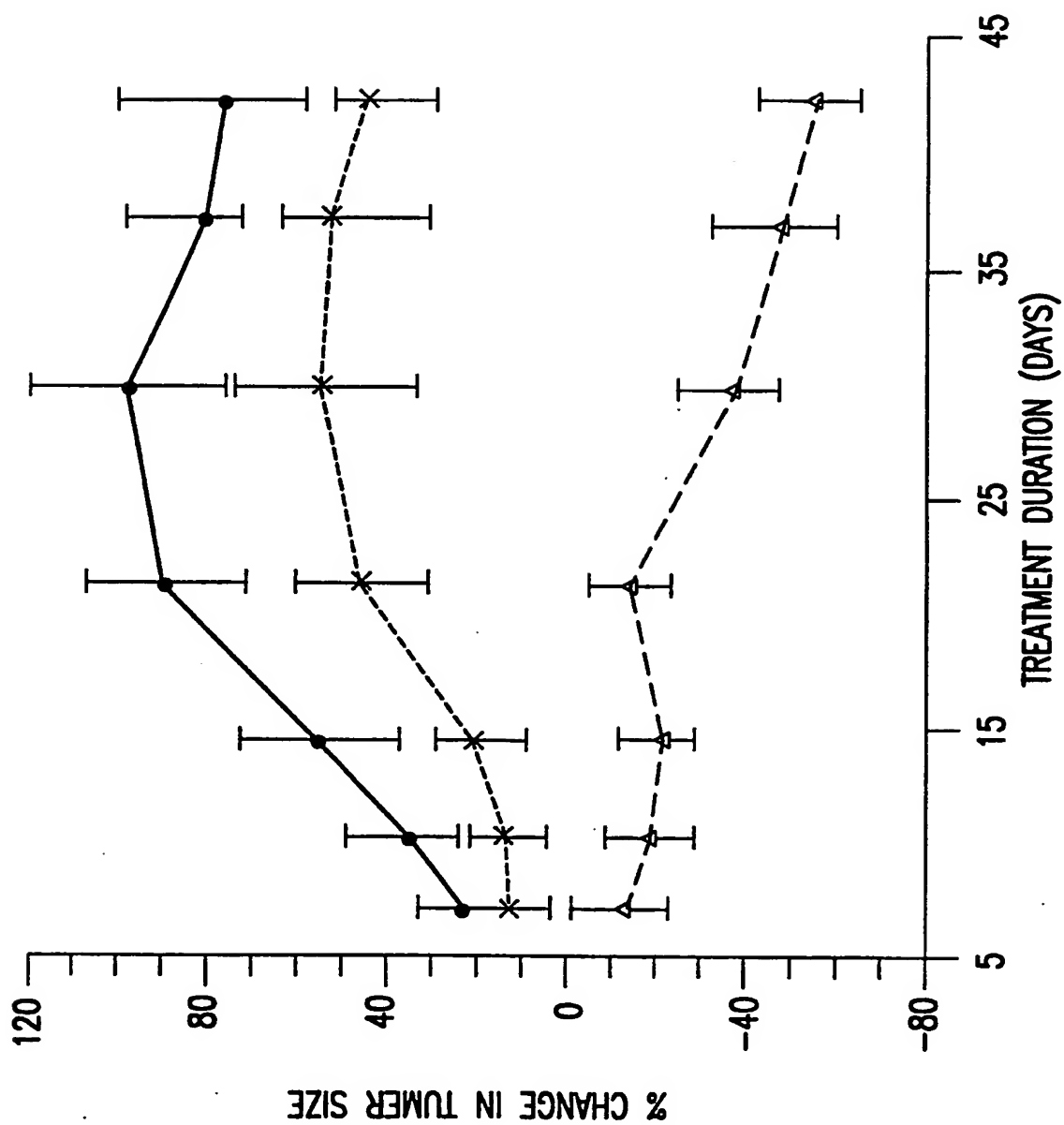


FIG. 4

5/5

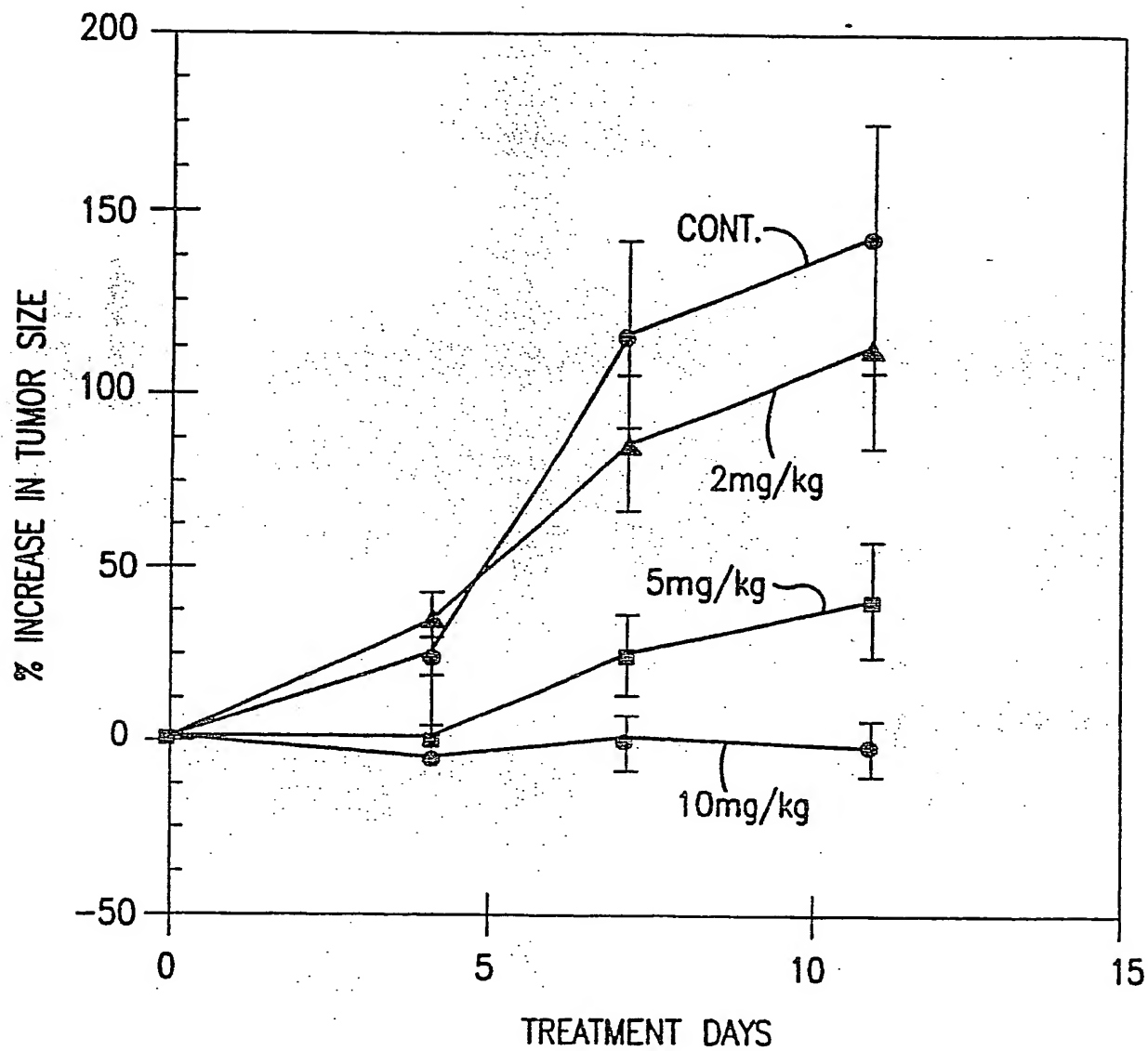


FIG.5